*Environ. Sci. Technol.* **2005**, *39,* 3034−3039

# Methylmercury in Mosquitoes Related to Atmospheric Mercury Deposition and Contamination

CHAD R. HAMMERSCHMIDT* AND WILLIAM F. FITZGERALD

*Department of Marine Sciences, University of Connecticut, Groton, Connecticut 06340*

A connection between loadings of inorganic Hg, especially from the atmosphere, and accumulation of methylmercury (MeHg) in aquatic biota has not been firmly established. Mosquitoes (Diptera: Culicidae) may be a useful indictor of Hg contamination or MeHg accumulation in aquatic ecosystems because they have aquatic life stages, and their ubiquitous distribution permits sampling across wide ranges of climate, biological productivity, and atmospheric Hg deposition. We examined MeHg in adult mosquitoes from subtropical (Florida), maritime (California), continental (Michigan), and arctic (Alaska) regions of North America that span a range in wet atmospheric Hg deposition (1.5−15 $\mu$g $m^{-2}$ $y^{-1}$). More than 90% of the Hg in mosquitoes was MeHg, and concentrations varied among locations. Levels of MeHg differed among mosquito species at six sites in northwest Florida (*Ochlerotatus atlanticus* < *Culex nigripalpus* < *Anopheles crucians*); this may be related to differences in biogeochemical characteristics of the aquatic habitat that affect dietary accumulation of MeHg during the larval stage. Mosquito MeHg was related positively to wet atmospheric Hg deposition among locations where atmospheric deposition is the principal source of Hg, and it was greatly enhanced in Hg-polluted environs near the Sulphur Bank Mine in Lake County, California. These results suggest that MeHg in mosquitoes may be a useful and sensitive indicator of Hg loadings to aquatic systems, including that derived from atmospheric deposition.

## Introduction

Accumulation of methylmercury (MeHg) in biota is a primary toxicological concern related to mercury in the environment. MeHg is produced from inorganic Hg by sulfate-reducing bacteria in aquatic systems (*1*, *2*) and it bioaccumulates in aquatic food webs (*3*). Loadings of inorganic Hg are a major control on the microbial production of MeHg (*4*−*6*), and atmospheric deposition is the principal source of Hg in most aquatic systems (*7*). However, a connection between inputs of inorganic Hg, especially from the atmosphere, and the accumulation of MeHg in aquatic biota has not been firmly established (*8*).

We used adult mosquitoes (Diptera: Culicidae) as biological indicators of MeHg accumulation in aquatic systems. Mosquitoes were selected because they are ubiquitous, easily collected, ecologically important, have aquatic life stages, and may be sensitive to natural and anthropogenic sources of Hg. Mosquitoes undergo four successive stages of development: egg, larva, pupa, and adult. The larval and pupal stages are exclusively aquatic, and they typically inhabit shallow wetlands and pools (*9*, *10*). All mosquito growth occurs during the larval stage (*9*, *10*) while feeding on microorganisms (i.e., bacteria, zooplankton, algae) and particulate organic detritus (*11*). If accumulation during the aquatic life stages were the primary source of Hg in mosquitoes, then the adult insects may provide a simple and useful measure of local Hg contamination. The objective of this work was to assess the potential utility of MeHg in adult mosquitoes as a biological indicator of Hg loadings and MeHg accumulation in aquatic ecosystems.

* Corresponding author phone: (860)405-9161; fax: (860)405-9153; e-mail: chad.hammerschmidt@uconn.edu.

## Experimental Section

**Mosquitoes.** Adult female mosquitoes were sampled with the assistance of local entomologists and mosquito control specialists from five locations in North America (Figure 1) between July 17 and September 21, 2003. These locations were selected to span ranges of climate (i.e., subtropical, continental, maritime, arctic), biological productivity, and atmospheric Hg deposition—factors that may affect the exposure of aquatic food webs to MeHg and inorganic Hg. Host-seeking adult female mosquitoes were captured with $CO_2$-baited traps (*12*) that were set for one to several days prior to collection. Adult mosquitoes in Arctic Alaska were attracted with $CO_2$ and netted by hand. Mosquitoes from each site were identified and sorted to the level of genus or species, packaged in new or acid-cleaned plastic containers, and promptly transported to the University of Connecticut, where they were stored frozen until lyophilization.

Adult mosquitoes were trapped at more than one site at four of the geographic locations (Table 1). The sites were selected by collaborating investigators knowledgeable of local mosquito breeding habitats and who provided samples from their own research and monitoring efforts. The eight sites in Bay County, FL, span a 20-km transect along the north Florida coast and are adjacent to wetlands. Two of the sites in Orange County, CA (OC-2 and OC-3) are suburban wetlands greater than 20 km distant, whereas the other two samples are composites of the same mosquito species from multiple urban sites scattered throughout Orange County. Mosquitoes were sampled at six riparian and woodland sites in Lake County, CA; five of these (sites CL-1 through CL-5) are within 10 km of Clear Lake and span an area of about 400 $km^2$, with an additional site (CL-6) about 30 km north of Clear Lake in the Snow Mountain Wilderness Area. Clear Lake is adjacent to one of California's largest Hg mines, the Sulfur Bank Mine, which operated periodically from 1865 to 1957 and is now designated as a Superfund site by the U.S. Environmental Protection Agency. The three sites in Midland County, MI, are in rural swamp and forested habitats and also covered an area of about 400 $km^2$. Adult mosquitoes were sampled at only one site, the southern shore of Toolik Lake, in the tundra of Arctic Alaska.

**MeHg Extraction and Analysis.** Subsamples (4−24 mg) of intact, lyophilized adult mosquitoes were weighed (±0.1 mg), counted, transferred to acid-cleaned 15-mL centrifuge tubes, and digested with 3.5−7.0 mL of 4.57 M $HNO_3$ in a covered (steamy) 60 °C water bath for 12 h. This extraction method quantitatively releases MeHg and inorganic Hg species from biological tissue, and its simple matrix is used easily with conventional purge-and-trap techniques that derivatize sample MeHg with sodium tetraethylborate. MeHg in the digestates was determined by gas-chromatographic cold-vapor atomic fluorescence spectroscopy (CVAFS) with analytical techniques described elsewhere (*13*, *14*). Briefly,


10.1021/es0485107 CCC: $30.25

Published on Web 03/12/2005

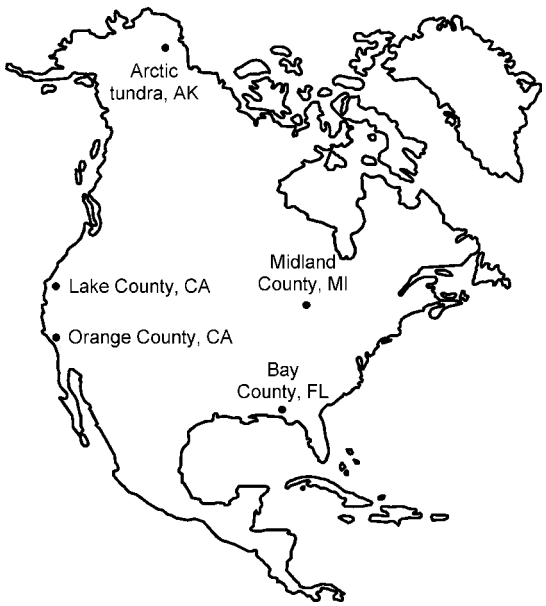


**FIGURE 1. Locations where adult mosquitoes were sampled in North America during the summer of 2003.**

a 100−1000 $\mu$L aliquot of the 4.57 M $HNO_3$ digestate was added to reagent-grade water (nominal resistance, 18.2 MΩ-cm) in a sparging flask, the acidity was neutralized with KOH, and the pH was adjusted to about 4.9 with 2 M acetate buffer. Sodium tetraethylborate (200 $\mu$L of 1% wt:vol solution) was added to the solution to derivatize the Hg species, and the volatile ethyl derivatives were purged from solution with $N_2$ and concentrated on Tenax. Ethylated derivatives of Hg species were thermally desorbed from the Tenax, separated by isothermal gas chromatography, decomposed pyrolytically, and detected with flow-injection CVAFS (*15*).

The accuracy of our MeHg determinations was estimated by analyses of (1) procedural blanks and calibration standards taken through the digestion process, (2) certified reference materials from the National Research Council of Canada, TORT-2 (lobster hepatopancreas) and DOLT-2 (dogfish liver), (3) replicate aliquots from the same digestate (i.e., analytical precision), (4) replicate subsamples of mosquitoes from the same parent sample (i.e., method precision/natural variability among organisms), and (5) subsamples of mosquitoes with known additions of MeHg. Sample MeHg was measured after calibration with procedural standards, typically a blank and three standard solutions taken through the digestion procedure; calibrations always yielded standard regressions with coefficients of determination greater than 0.995. All analyses of MeHg in the reference materials were within their certified ranges; our mean-measured concentration of MeHg in TORT-2 was 154 ng $g^{-1}$ dry weight (certified range, 139−165 ng $g^{-1}$) and that in DOLT-2 was 683 ng $g^{-1}$ (certified range, 640−746 ng $g^{-1}$). The mean recovery of MeHg from procedurally spiked digestates of mosquito was 104% (range, 96−111%). Analytical precision of MeHg determinations from these digestates averaged 5.0% relative standard deviation (RSD), which is similar to the precision of MeHg measurements in other matrixes (e.g., sediments, fish muscle) with the same analytical system (*6*, *14*, *15*). However, the mean method precision for this study (21.3% RSD) was considerably greater than the analytical precision. This indicates some heterogeneity in the MeHg content among mosquitoes in each sample (discussed below).

**Total Hg Extraction and Analysis.** $HNO_3$ digestates for MeHg analysis also were used for determination of total Hg, which includes both MeHg and organic and inorganic complexes of $Hg^{2+}$, after treatment with a strong chemical oxidant. The ability to use the same tissue aliquot for measurement of both MeHg and total Hg is especially important for biological samples that are limited in size (e.g., insects, fish eggs), and it minimizes some of the random error (e.g., sample mass determinations, within-sample heterogeneity) associated with analysis of each Hg fraction in separate aliquots of the same parent material (*16*). Aliquots of 4.57 M $HNO_3$ digestates were transferred to acid-cleaned 15-mL centrifuge tubes, diluted with reagent-grade water, and oxidized with 0.4−0.6 mL of BrCl solution (*17*). The volume of $HNO_3$ digestate transferred and its degree of dilution were dependent on the expected amount of total Hg in the diluted sample. The volume of BrCl solution added was a function of the organic content in the diluted extract. A sample with sufficient BrCl will retain a yellow-orange hue throughout the total Hg digestion period. BrCl-oxidized digestates were analyzed for total Hg by dual-Au amalgamation CVAFS (*18*, *19*). Total Hg analyses were calibrated with $Hg^0$ standards removed from the headspace over pure liquid (*20*) and verified by comparison to analyses of aqueous $Hg^{2+}$ solutions traceable to the U.S. National Institute of Standards and Technology (NIST). Standard solutions of MeHg also were calibrated, after BrCl oxidation, by comparison to NIST-traceable $Hg^{2+}$ solutions and $Hg^0$ standards. Calibrations for total Hg analysis, typically five $Hg^0$ standards, yielded linear regressions with coefficients of determination greater than 0.995. Recovery of added $Hg^{2+}$ averaged 94% (range, 92−96%) compared to $Hg^0$ standards. Similar to our determinations of MeHg in TORT-2 and DOLT-2, all analyses of total Hg in these reference materials also were within their certified ranges and near the certified means. Our mean-measured concentration of total Hg in TORT-2 was 254 ng $g^{-1}$ dry weight (certified range, 210−330 ng $g^{-1}$) and that in DOLT-2 was 2027 ng $g^{-1}$ (certified range, 1860−2420 ng $g^{-1}$). The analytical precision of total Hg determinations averaged 1.6% RSD, and the method precision of total Hg measurements was similar to that of MeHg analyses. The estimated detection limit for MeHg and total Hg in a 15-mg sample of lyophilized mosquito was about 0.4 ng $g^{-1}$.

**Wet Atmospheric Hg Deposition.** Estimates for wet atmospheric deposition of total Hg are from several sources at or near our mosquito collection locations. These include Mercury Deposition Network (MDN; *21*) stations AL24 (coastal Alabama, 2002−2003 data; for nearby Bay County, FL), CA97 (Mendocino County, CA, October 20, 1998−October 19, 2000; for adjacent Lake County), and CA72 (San Jose, CA, 2000−2003; for Orange County, CA). There is no MDN station near Orange County, CA, but recent work by Steding and Flegal (*22*) has indicated that the wet depositional flux of Hg measured in San Jose is typical for all of coastal California, which would include Orange County. Wet deposition of total Hg in Midland County, MI, was estimated as the average between measured annual fluxes at Pellston, MI, located northwest of Midland County, and at Dexter, MI, an equidistant site to the southeast (March 1992−March 1994; *23*). Wet Hg deposition in Arctic Alaska was determined from our measurements at Toolik Field Station (July 2000−July 2002; *24*), the same location where arctic mosquitoes were sampled. The most recent Hg deposition results were used for a location when data from 2003 were not available. Standard errors were calculated from interannual differences in Hg deposition at each location.

## Results and Discussion

**Mosquito MeHg.** MeHg in adult mosquitoes ranged from 10 to 480 ng $g^{-1}$ dry weight among samples (i.e., same genera, same site) and varied among our sampling locations in North America (Table 1). Concentrations of MeHg in adult mosquitoes are similar to those in other aquatic insects and invertebrates, including *Hexagenia* mayfly nymphs (30−130

**TABLE 1. Summary Information for Locations Where Mosquitoes Were Sampled for MeHg Analysis**

| location | site ID | site description | site location | mosquito species[a] | mosquito MeHg (ng $g^{-1}$ dry wt)[b] | wet atmospheric Hg deposition ($\mu g\ m^{-2}\ y^{-1}$)[c] |
|---|---|---|---|---|---|---|
| Bay County, FL | FL-1 | Frank Brown Park | 30.233° N, 85.874° W | multiple | 101 ± 21[d] | 15.0 ± 0.6 |
| | FL-2 | Pearl Avenue | 30.231° N, 85.885° W | multiple | 113 ± 13[d] | |
| | FL-3 | Lakeside | 30.225° N, 85.879° W | multiple | 101 ± 32[d] | |
| | FL-4 | Ed's Sheds | 30.190° N, 85.777° W | multiple | 106 ± 20[d] | |
| | FL-5 | treatment plant | 30.218° N, 85.852° W | multiple | 131 ± 37[d] | |
| | FL-6 | 2020 Place | 30.165° N, 85.756° W | multiple | 237 ± 76[d] | |
| | FL-7 | Lairds | 30.143° N, 85.714° W | *Cx. erraticus* | 97 ± 16 | |
| | FL-8 | Bayside | 30.202° N, 85.761° W | *Oc. atlanticus* | 112 ± 16 | |
| Arctic Alaska | AK | Toolik Field Station | 68.62° N, 149.60° W | *Ochlerotatus* sp. | 23 ± 1 | 1.5 ± 0.6 |
| Lake County, CA | CL-1 | Sulphur Bank Mine tailings | 39.002° N, 122.667° W | *Cx. tarsalis* | 107 ± 13 | 3.5 ± 0.01 |
| | CL-2 | M&M Resort, Clearlake Oaks | 39.016° N, 122.665° W | *Cx. tarsalis* | 163 ± 49 | |
| | CL-3 | Willow Point, Lakeport | 39.040° N, 122.911° W | *Cx. tarsalis* | 480 ± 235 | |
| | CL-4 | Anderson Marsh | 38.920° N, 122.630° W | *Cx. tarsalis* | 136 ± 46 | |
| | CL-5 | Starke Ranch | 38.919° N, 122.774° W | *Cx. tarsalis* | 244 ± 11 | |
| | CL-6 | Snow Mt. Wilderness | 39.345° N, 122.753° W | *Cx. tarsalis* | 19 ± 2 | |
| Orange County, CA | OC-1 | multiple suburban sites | | *Cx. quinquefasciatus* | 19 | 3.1 ± 0.5 |
| | OC-2 | Robinson Ranch Marsh | | *Cx. erythrothorax* | 12 ± 1 | |
| | OC-3 | UC-Irvine Ecological Pres. | | *Cx. erythrothorax* | 10 ± 1 | |
| | OC-4 | multiple suburban sites | | *Cx. tarsalis* | 36 | |
| Midland County, MI | MI-1 | ball diamonds, Coleman | 43.75° N, 84.57° W | *Ae. vexans* | 87 | 7.3 ± 1.6 |
| | MI-2 | Arbutus Bog | 43.68° N, 84.45° W | *Co. perturbans* | 24 ± 2 | |
| | MI-3 | Kawkawlin Flooding Area | 43.80° N, 84.27° W | *Anopheles* sp. | 47 | |

[a] Genus names are abbreviated (*Cx.* = *Culex*; *Oc.* = *Ochlerotatus*; *Ae.* = *Aedes*; *Co.* = *Coquillettidia*). [b] Mean (±1 SE) for multiple analyses of the same mosquito sample (i.e., same genera, same site). [c] Atmospheric Hg deposition values (±1 SE) are described and referenced in the Experimental Section. [d] Mean (±1 SE) for multiple mosquito species at the same site. Concentrations in individual species at these locations are shown in Figure 4.

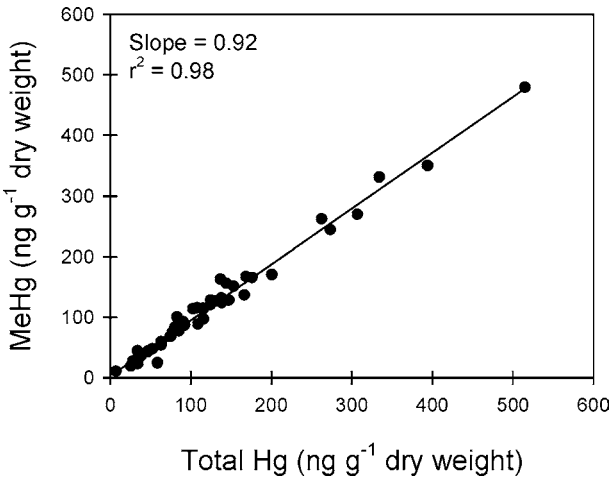


**FIGURE 2. Relation between MeHg and total Hg in adult mosquitoes from various locations in North America.**

ng $g^{-1}$ dry weight; *25*), zooplankton (10−290 ng $g^{-1}$; *26*, *27*), *Simulium* blackflies (7−640 ng $g^{-1}$; *28*), and various insect taxa (10−800 ng $g^{-1}$; *29*−*31*). In general, mosquito MeHg was lowest in Orange County, CA, (mean, 19 ng $g^{-1}$) and Arctic Alaska (23 ng $g^{-1}$) and greatest in Lake County, CA (192 ng $g^{-1}$), regardless of mosquito species. Loadings of inorganic Hg, among other environmental factors, are a major control on the in-situ bacterial production of MeHg (*4*−*6*), and atmospheric deposition is the principal source of Hg in most aquatic systems (*7*). Thus, the geographical variation in mosquito MeHg bioaccumulation may reflect local/regional differences in the degree of inorganic Hg contamination among these sampling locations.

**Hg Speciation in Adult Mosquitoes.** Most of the Hg in mosquitoes was MeHg (Figure 2), the bioaccumulative and toxic form of Hg. On average, MeHg comprised 92% (slope of regression) of the total Hg in adult mosquitoes. This means that mosquitoes preferentially accumulate MeHg relative to inorganic and organic complexes of $Hg^{2+}$. The fraction of total Hg as MeHg in adult mosquitoes of this study was relatively high compared to that in other invertebrates presumably occupying a comparable trophic level, including zooplankton (10−80%; *26*, *27*, *31*) and other aquatic insects (10−85%; *25*, *29*, *31*). Also, this fraction was only a little less than that typically found in fish (>95% MeHg; *16*). Not included in the regression analysis in Figure 2 were mosquitoes collected at site CL-1 in Lake County; Hg in these mosquitoes was only 54 ± 12% MeHg. This sampling site was about 15 m from the tailings of the Sulphur Bank Mine. The unusually low percentage of total Hg as MeHg in mosquitoes at this site suggests some uptake of inorganic Hg from the heavily polluted environs near the mine tailings. Lake sediments near the mine, for example, can contain greater than 100 $\mu g\ g^{-1}$ total Hg (*32*), a concentration $10^2$−$10^3$ greater than those typically found in most lacustrine deposits (*8*).

**Interspecies Variation in Mosquito MeHg.** We found interspecies differences in the MeHg content of mosquitoes from Bay County, FL, where multiple species of mosquitoes were collected at each of six sites (Table 1; Figure 3). Three species of mosquito were present in most samples collected from Bay County: *Ochlerotatus atlanticus*, *Culex nigripalpus*, and *Anopheles crucians*. At most of these sites, a similar distribution in mosquito MeHg concentration was apparent: *Oc. atlanticus* < *Cx. nigripalpus* < *An. crucians*. From these results, though limited in sample replication and statistical significance, it appears that certain mosquito species either may accumulate MeHg more efficiently than others (e.g., differences in larval diet) or species-specific preferences in breeding habitats may confer variations in MeHg production and accumulation within a general area.

**Source of MeHg in Mosquitoes.** We did not directly assess the accumulation of MeHg in mosquitoes during each of their four life stages; however, it can be reasonably inferred that dietary exposure during the aquatic larval stage is the principal source of MeHg in these insects. All somatic growth

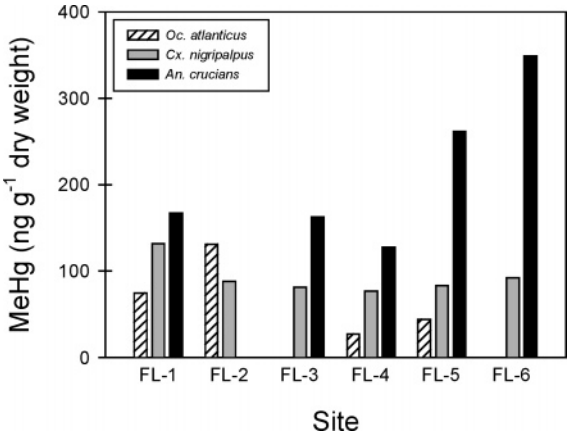


**FIGURE 3. Interspecies differences in the MeHg content of adult mosquitoes from sites in Bay County, FL, where more than one species was collected.**

of mosquitoes results from feeding during the larval stage (*9*, *10*). Studies with both fish (*33*) and zooplankton (*34*) have shown that diet is the primary source of MeHg in these organisms, and by extension, diet should be the principal exposure pathway for other heterotrophs. If uptake from water during the immature aquatic life stages were the major source of MeHg in mosquitoes, then their MeHg content should be similar to that of phytoplankton, which bioconcentrate waterborne Hg species (*35*). However, the fraction of total Hg as MeHg in mosquitoes (~90%; Figure 2) is much greater than that in phytoplankton (<20%; *26*). The relatively high percentage of total Hg as MeHg in mosquitoes indicates biomagnification of MeHg through dietary trophic transfer (*3*). This could occur only during the aquatic larval stage when mosquitoes forage on microorganisms and detritus, although a limited amount of MeHg may be accumulated as adults in terrestrial habitats.

Occasional meals of sugar (i.e., nectar) and blood should have minor influence on the MeHg content of adult mosquitoes, especially for those collected with $CO_2$-baited traps. Most (>80%) female mosquitoes collected with $CO_2$-baited traps are host-seeking for the first time and have not taken a blood meal (*36*), and of those that have fed previously, only a small fraction have had more than one meal (*37*, *38*). Adult mosquitoes consume plant sugar to provide energy for survival, flight, and mating (*39*), and females ingest blood principally for egg production (*10*). Sugar and blood meals can vary in size (*39*); blood meals range from a trace amount to 5 mg (*10*). We estimate that each blood meal contains about 2 pg of MeHg, using an average blood meal size of 2 mg wet weight (about the same mass as adult mosquitoes in this study) and an average concentration of MeHg in human and avian blood of about 1 $\mu g\,L^{-1}$ (*40*, *41*). This amount is less than 5% of the mean MeHg burden of adult mosquitoes in this study (53 pg). The blood meal, however, is used primarily to nourish developing oocytes and not for somatic growth (*10*, *39*). Furthermore, and if all of the MeHg from a blood meal were assimilated by the adult and not transferred to eggs, the estimated MeHg contribution from a single blood meal would be comparable to the analytical error of our MeHg determinations (5% RSD). Sugar meals may be more frequent than blood feeding (*39*), yet the MeHg content of nectar, on the basis of average levels of MeHg in fruits (*8*), is about $10^3$ less than that of blood. Hence, the MeHg content of adult mosquitoes should reflect their dietary exposure to the contaminant as larvae in aquatic systems, which is influenced by environmental factors affecting the production and bioaccumulation of MeHg, including atmospheric deposition of inorganic Hg.

While we do not know the biogeochemical characteristics of the specific breeding habitats of the mosquitoes collected for this study, we posit that interspecies variations in the MeHg content of adult mosquitoes are due to differences in breeding habitats among species. Of the three major mosquito species collected in Bay County, FL, *An. crucians* contained more MeHg than the others (Figure 3), and this species, unlike *Oc. atlanticus* and *Cx. nigripalpus*, generally prefers acidic water for its larval habitat (*9*). Surface water acidity promotes the bacterial production of MeHg (*4*, *42*) and its accumulation in emergent insects (*28*). This could explain relatively enhanced levels of MeHg in *An. crucians* as compared to the other mosquito species. Alternatively, interspecies differences in larval feeding modes and microhabitats (*11*), as well as the length of larval development (*43*), could contribute to variable MeHg accumulation among mosquito species at a site.

**Within-Sample MeHg Variation.** Multiple subsample digestions and analyses were conducted for mosquito samples having greater than 40 individuals. Although a single mosquito contained enough MeHg for reliable chemical analysis, more than 10 were needed for accurate gravimetric measurement. Variability in MeHg concentration among mosquitoes from the same sample (mean, 23.1% RSD) was much greater than our analytical precision (mean, 5.0% RSD), indicating some natural variation in MeHg among mosquitoes sampled at a particular site. Unlike analytical precision, which typically increases with sample concentration, the relative precision of methodically replicated subsamples decreased with increasing mosquito MeHg content. The procedural precision for analyses of mosquitoes having less than 120 ng $g^{-1}$ dry weight MeHg averaged 12.3% RSD (range, 4.8−21.6%), whereas that of samples having greater than this concentration averaged 31.6% RSD (range, 6.4−49.0%).

Most of the MeHg in adult mosquitoes presumably is accumulated during the larval stage, and consequently, differences in the MeHg content of adult mosquitoes in a sample (i.e., same genera, same collection site) can result from local variations in larval exposure. Adult mosquitoes can disperse up to 40 km, but the distance is species dependent and typically much less (*44*). Thus, adults collected at a particular site provide an integrated assessment of local MeHg bioaccumulation. Little variation in mosquito MeHg would be expected at sites where Hg loadings and MeHg bioaccumulation are similar throughout the surrounding area. Atmospheric deposition is the presumed dominant and spatially ubiquitous source of Hg contamination in the tundra of Arctic Alaska, for example, and the variation in MeHg among mosquitoes at this location was relatively low (about 10% RSD). At sites near localized sources of Hg or with enhanced MeHg bioaccumulation, more variability in mosquito MeHg would be expected because insects captured at these sites have dispersed from both relatively low and high exposure locales. This may explain the high variability in MeHg for *Culex tarsalis* collected at sites in the vicinity of Clear Lake and the Sulphur Bank Mine (sites CL-1 through CL-5; Table 1). The mean concentration of MeHg in these mosquitoes was relatively high (226 ng $g^{-1}$ dry weight), and so was the variability among replicate subsamples (mean intrasample variation, 33.5% RSD).

**Geographical Variation in Mosquito MeHg.** Mean levels of MeHg in mosquitoes were related positively to wet atmospheric deposition of total Hg (Figure 4). The number of sampling locations is limited, but the range in wet Hg deposition (1.5−15 $\mu g\,m^{-2}\,y^{-1}$) is typical for most of North America (*8*). This, and the relationship in Figure 4, implies that wet atmospheric Hg deposition may be a major factor affecting the production and bioaccumulation of MeHg in aquatic systems. This relationship is striking given the large geographical and climatological range of the sampling

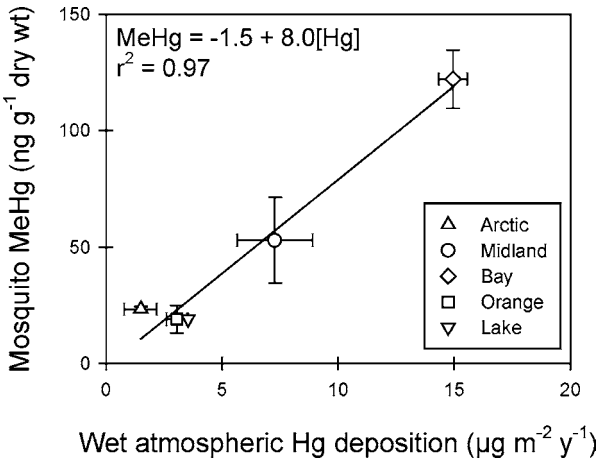


**FIGURE 4. Relation between the mean concentration of MeHg in adult mosquitoes and wet atmospheric deposition of total Hg (±1 SE) measured at representative sites near the mosquito collection locations (Arctic tundra, AK; Midland County, MI; Bay County, FL; Orange County, CA; Lake County, CA). Not included in the regression analysis are mosquitoes from sites CL-1 through CL-5 in Lake County, which have suspected Hg enrichment from the Sulfur Bank Mine. The mean MeHg content of these insects is out of range and not shown (mosquito MeHg, 226 ± 67 ng $g^{-1}$ dry weight; Hg deposition, 3.5 $\mu g$ $m^{-2}$ $y^{-1}$).**

locations and that numerous other environmental factors (e.g., pH, organic matter, temperature, sulfur chemistry) can affect the bacterial transformation of inorganic Hg to MeHg (*4*−*6*, *42*). Moreover, the relationship in Figure 4 suggests that atmospheric deposition of reactive gaseous Hg (*45*), which is not collected with wet deposition, either is relatively proportional to wet atmospheric fluxes or may not considerably affect the net production and bioaccumulation of MeHg at *most* locations.

Mosquitoes collected at sites within 10 km of Clear Lake in Lake County, CA (sites CL-1 through CL-5; Table 1) were outliers to the relationship shown in Figure 4 and were not included in the regression analysis. The anomalously high concentrations of MeHg in mosquitoes at these sites may be explained by locally enhanced levels of inorganic Hg from non-atmospheric sources, namely, the Sulphur Bank Mine that is adjacent to Clear Lake. Emissions of gaseous Hg from the mine (*46*), and leaching of mining remnants after its operation (*47*), have contaminated the watershed with inorganic Hg. This has resulted in enhanced levels of MeHg in the food web (*32*, *48*). Adult *Cx. tarsalis* sampled at sites in the vicinity of Clear Lake (i.e., sites CL-1 through CL-5) have elevated concentrations of MeHg (mean, 226 ng $g^{-1}$ dry weight) compared to those at a reference site in Lake County (site CL-6, 19 ng $g^{-1}$; Table 1) that is more than 40 km from the mine and about 30 km north of Clear Lake. Wet atmospheric deposition is the presumed dominant source of Hg at site CL-6, and the MeHg content of mosquitoes there is in agreement with the relationship in Figure 4. Although mosquitoes sampled at sites CL-1 through CL-5 are outliers to the relationship in Figure 4, they further illustrate the sensitivity of mosquito MeHg to local Hg contamination.

We found that MeHg concentrations in North American mosquitoes were related to loadings of inorganic Hg, mostly from wet atmospheric deposition. This may be surprising given the many biogeochemical factors that can affect both the transformation of inorganic Hg to MeHg and the subsequent bioaccumulation of MeHg. Although the number of sampling locations was small, this relationship suggests that the supply of inorganic Hg to freshwater systems may be a major factor influencing the accumulation of MeHg in mosquitoes, and by extension, other organisms. Moreover, MeHg in mosquitoes may provide a simple but useful biological measure of atmospheric Hg deposition and food web MeHg accumulation. Clearly, and given the limited number of sampling locations in this study, the promising potential of this ubiquitous bioindicator species merits further investigation.

## Acknowledgments

We are grateful to the following researchers who provided mosquitoes for this study: David Woodward (Lake County Vector Control District, CA), Thomas Wilmot (Midland County Mosquito Control, MI), Robert Cummings (Orange County Vector Control District, CA), and Dale Martin (Beach Mosquito Control District, Panama City Beach, FL). Helpful reviews of earlier drafts of the manuscript were provided by David Woodward, Robert Cummings, Kristopher Rolfhus, and three anonymous reviewers. This study was supported by the National Science Foundation−Office of Polar Programs (9908895) and the U.S. Environmental Protection Agency through a STAR graduate fellowship (91591801). The work, however, does not necessarily reflect the views of the U.S. EPA, and no official endorsement should be inferred.

## Literature Cited

(1) Compeau, G.; Bartha, R. Sulfate-reducing bacteria: Principal methylators of mercury in anoxic estuarine sediment. *Appl. Environ. Microbiol.* **1985**, *50*, 498−502.

(2) Gilmour, C. C.; Henry, E. A.; Mitchell, R. Sulfate stimulation of mercury methylation in freshwater sediments. *Environ. Sci. Technol.* **1992**, *26*, 2281−2288.

(3) Wiener, J. G.; Krabbenhoft, D. P.; Heinz, G. H.; Scheuhammer, A. M. Ecotoxicology of mercury. In *Handbook of Ecotoxicology*; Hoffman, D. J., Rattner, B. A., Burton, G. A., Jr., Cairns, J., Jr., Eds.; Lewis Publishers: Boca Raton, FL, 2003; pp 409−463.

(4) Gilmour, C. C.; Henry, E. A. Mercury methylation in aquatic systems affected by acid deposition. *Environ. Pollut.* **1991**, *71*, 131−169.

(5) Benoit, J. M.; Gilmour, C. C.; Heyes, A.; Mason, R. P.; Miller, C. L. Geochemical and biological controls over methylmercury production and degradation in aquatic ecosystems. In *Biogeochemistry of Environmentally Important Trace Elements*; Cai, Y., Braids, O. C., Eds.; American Chemical Society: Washington, DC, 2003; pp 262−297.

(6) Hammerschmidt, C. R.; Fitzgerald, W. F. Geochemical controls on the production and distribution of methylmercury in near-shore marine sediments. *Environ. Sci. Technol.* **2004**, *38*, 1487−1495.

(7) Fitzgerald, W. F.; Engstrom, D. R.; Mason, R. P.; Nater, E. A. The case for atmospheric mercury contamination in remote areas. *Environ. Sci. Technol.* **1998**, *32*, 1−7.

(8) *Mercury Study Report to Congress*; U.S. Environmental Protection Agency: Washington, DC, 1997.

(9) Carpenter, S. J.; LaCasse, W. J. *Mosquitoes of North America: North of Mexico*; University of California Press: Berkeley, CA, 1955.

(10) Gillett, J. D. *The Mosquito: Its Life, Activities, and Impact on Human Affairs*; Doubleday: Garden City, NY, 1972.

(11) Merritt, R. W.; Dadd, R. H.; Walker, E. D. Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annu. Rev. Entomol.* **1992**, *37*, 349−367.

(12) Sudia, W. D.; Chamberlain, R. W. Battery-operated light trap, an improved model. *Mosquito News* **1962**, *22*, 126−129.

(13) Bloom, N. S. Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapour atomic fluorescence detection. *Can. J. Fish. Aquat. Sci.* **1989**, *46*, 1131−1140.

(14) Hammerschmidt, C. R.; Fitzgerald, W. F. Formation of artifact methylmercury during extraction from a sediment reference material. *Anal. Chem.* **2001**, *73*, 5930−5936.

(15) Tseng, C.-M.; Hammerschmidt, C. R.; Fitzgerald, W. F. Determination of methylmercury in environmental matrixes by on-line flow injection and atomic fluorescence spectrometry. *Anal. Chem.* **2004**, *76*, 7131−7136.

(16) Bloom, N. S. On the chemical form of mercury in edible fish and marine invertebrate tissue. *Can. J. Fish. Aquat. Sci.* **1992**, *49*, 1010−1017.

(17) Bloom, N. S.; Crecelius, E. A. Determination of mercury in seawater at sub-nanogram per liter levels. *Mar. Chem.* **1983**, *14*, 49−59.
(18) Fitzgerald, W. F.; Gill, G. A. Subnanogram determination of mercury by two-stage gold amalgamation applied to atmospheric analysis. *Anal. Chem.* **1979**, *51*, 1714−1720.
(19) Bloom, N. S.; Fitzgerald, W. F. Determination of mercury species at the picogram level by low-temperature gas chromatography with cold-vapor atomic fluorescence detection. *Anal. Chim. Acta* **1988**, *208*, 151−161.
(20) Gill, G. A.; Fitzgerald, W. F. Picomolar mercury measurements in seawater and other materials using stannous chloride and two-stage amalgamation with gas phase detection. *Mar. Chem.* **1987**, *20*, 227−243.
(21) National Atmospheric Deposition Program: Mercury Deposition Network. http://nadp.sws.uiuc.edu/mdn/ (accessed August 11, 2004).
(22) Steding, D. J.; Flegal, A. R. Mercury concentrations in coastal California precipitation: Evidence of local and trans-Pacific fluxes of mercury to North America. *J. Geophys. Res.* **2002**, doi: 10.1029.2002JD002081.
(23) Hoyer, M.; Burke, J.; Keeler, G. Atmospheric sources, transport and deposition of mercury in Michigan: Two years of event precipitation. *Water, Air, Soil Pollut.* **1995**, *80*, 199−208.
(24) Fitzgerald, W. F.; Engstrom, D. R.; Lamborg, C. H.; Tseng, C.-M.; Balcom, P. H.; Hammerschmidt, C. R. Modern and historic atmospheric mercury fluxes in northern Alaska: Global sources and arctic depletion. *Environ. Sci. Technol.* **2005**, *39*, 557−568.
(25) Naimo, T. J.; Wiener, J. G.; Cope, W. G.; Bloom, N. S. Bioavailability of sediment-associated mercury to *Hexagenia* mayflies in a contaminated floodplain river. *Can. J. Fish. Aquat. Sci.* **2000**, *57*, 1092−1102.
(26) Watras, C. J.; Bloom, N. S. Mercury and methylmercury in individual zooplankton: Implications for bioaccumulation. *Limnol. Oceanogr.* **1992**, *37*, 1313−1318.
(27) Paterson, M. J.; Rudd, J. W. M.; St. Louis, V. Increases in total and methylmercury in zooplankton following flooding of a peatland reservoir. *Environ. Sci. Technol.* **1998**, *32*, 3868−3874.
(28) Harding, K. M.; Dillon, P.; Gowland, J. Mercury concentrations in blackflies *Simulium* spp (Diptera, Simuliidae) from softwater streams in Ontario, Canada. *RMZ-Mater. Geoenviron.* **2004**, *51*, 1037−1040.
(29) Tremblay, A.; Cloutier, L.; Lucotte, M. Total mercury and methylmercury fluxes via emerging insects in recently flooded hydroelectric reservoirs and a natural lake. *Sci. Total Environ.* **1998**, *219*, 209−221.
(30) Hall, B. D.; Rosenberg, D. M.; Wiens, A. P. Methyl mercury in aquatic insects from an experimental reservoir. *Can. J. Fish. Aquat. Sci.* **1998**, *55*, 2036−2047.
(31) Gorski, P. R.; Cleckner, L. B.; Hurley, J. P.; Sierzen, M. E.; Armstrong, D. E. Factors affecting enhanced mercury bioaccumulation in inland lakes of Isle Royale National Park, USA. *Sci. Total Environ.* **2003**, *304*, 327−348.
(32) Suchanek, T. H.; Richerson, P. J.; Holts, L. J.; Lamphere, B. A.; Woodmansee, C. E.; Slotton, D. G.; Harner, E. J.; Woodward, L. A. Impacts of mercury on benthic invertebrate populations and communities within the aquatic ecosystem of Clear Lake, California. *Water, Air, Soil Pollut.* **1995**, *80*, 951−960.
(33) Hall, B. D.; Bodaly, R. A.; Fudge, R. J. P.; Rudd, J. W. M.; Rosenberg, D. M. Food as the dominant pathway of methylmercury uptake by fish. *Water, Air, Soil Pollut.* **1997**, *100*, 13−24.
(34) Tsui, M. T. K.; Wang, W. X. Uptake and elimination routes of inorganic mercury and methylmercury in *Daphnia magna*. *Environ. Sci. Technol.* **2004**, *38*, 808−816.
(35) Mason, R. P.; Reinfelder, J. R.; Morel, F. M. M. Uptake, toxicity, and trophic transfer of mercury in a coastal diatom. *Environ. Sci. Technol.* **1996**, *30*, 1835−1845.
(36) Morris, C. D.; Defoliart, G. R. Parous rates in mosquito populations. *J. Med. Entomol.* **1971**, *8*, 209−212.
(37) Forattini, O. P.; Kakitani, I., Massad, E.; Gomes, A. Studies on mosquitoes (Diptera: Culicidae) and anthropic environment: 1-Parity of blood seeking Anopheles (Kerteszia) in south-eastern Brasil. *Rev. Saúde Pública* **1993**, *27*, 1−8.
(38) Riesen, W. K.; Lothrop, H. D.; Hardy, J. L. Bionomics of *Culex tarsalis* (Diptera: Culicidae) in relation to arbovirus transmission in southeastern California. *J. Med. Entomol.* **1995**, *32*, 316−327.
(39) Foster, W. A. Mosquito sugar feeding and reproductive energetics. *Annu. Rev. Entomol.* **1995**, *40*, 443−474.
(40) Wayland, M.; Garcia-Fernandez, A. J.; Neugebauer, E.; Gilchrist, H. G. Concentrations of cadmium, mercury and selenium in blood, liver and kidney of common eider ducks from the Canadian arctic. *Environ. Monit. Assess.* **2001**, *71*, 255−267.
(41) Mahaffey, K. R.; Clickner, R. P.; Bodurow, C. C. Blood organic mercury and dietary mercury intake: National health and nutrition examination survey, 1999 and 2000. *Environ. Health Perspect.* **2004**, *112*, 562−570.
(42) Winfrey, M. R.; Rudd, J. W. M. Environmental factors affecting the formation of methylmercury in low pH lakes. *Environ. Toxicol. Chem.* **1990**, *9*, 853−869.
(43) Laird, M. *The Natural History of Larval Mosquito Habitats*; Academic Press: New York, 1988.
(44) Service, M. W. *Mosquito Ecology Field Sampling Methods,* 2nd ed.; Elsevier Applied Science: New York, 1993.
(45) Lindberg, S. E.; Stratton, W. J. Atmospheric mercury speciation: Concentrations and behavior of reactive gaseous mercury in ambient air. *Environ. Sci. Technol.* **1998**, *32*, 49−57.
(46) Nacht, D. M.; Gustin, M. S.; Engle, M. A.; Zehner, R. E.; Giglini, A. D. Atmospheric mercury emissions and speciation at the Sulphur Bank Mercury Mine Superfund site, northern California. *Environ. Sci. Technol.* **2004**, *38*, 1977−1983.
(47) Suchanek, T. H.; Mullen, L. H.; Lamphere, B. A.; Richerson, P. J.; Woodmansee, C. E.; Slotton, D. G.; Harner, E. J.; Woodward, L. A. Redistribution of mercury from contaminated lake sediment of Clear Lake, California. *Water, Air, Soil Pollut.* **1998**, *104*, 77−102.
(48) Elbert, R. A.; Anderson, D. W. Mercury levels, reproduction, and hematology in western grebes from three California lakes, USA. *Environ. Toxicol. Chem.* **1998**, *17*, 210−213.

*Received for review September 22, 2004. Revised manuscript received February 8, 2005. Accepted February 10, 2005.*

ES0485107